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Effect of protected organic acid blends on growth performance, nutrient digestibility and faecal micro flora in growing pigs

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This study was conducted to evaluate the effects of dietary supplementation of protected organic acid blends on growth performance, nutrient digestibility and faecal micro flora in growing pigs. A total of 100 crossed [(Landrace × Yorkshire) × Duroc] with an initial body weight (BW) of 23.4 ± 1.27 kg were used in a six-week trial. Pigs were randomly allocated into one of four treatments (five replications with five pigs per pen) in a randomly complete block design based on their BW. Treatments consisted of CON, basal diet; MC1, basal diet + protected organic acid 0.1%; MC2, basal diet + protected organic acid 0.2%; MC3, basal diet + protected organic acid 0.4%. BW and feed intake were measured initially and at the end of six weeks to determine average daily gain (ADG), average daily feed intake (ADFI) and gain/feed. The digestibility of nutrients and faecal micro flora was also assessed at the end of the experiment. The pigs fed MC2 diet showed greater ADG and G:F than pigs fed the control diet. Linear effects for ADG (linear, $P = 0.04$) as well as G:F (linear, $P = 0.03$) were observed. Dietary supplementation with 0.1%, 0.2% and 0.4% protected organic acid did not affect dry matter, N and energy digestibility. Supplementation of 0.2% protected organic acid increased ($P < 0.05$) faecal *Lactobacillus* population counts (linear, $P = 0.01$). Our result suggests that protected organic acid has the potential to enhance growth performance and improve microbial population in growing pigs.

Keywords: digestibility; growing pig; growth performance; micro flora; protected organic acids

1. Introduction

Following the ban of antibiotics as growth promoter due to bacterial resistance issues, a need for alternative method to improve growth and efficiency of pig production has been realised. This has prompted the scientific investigation of several feed additives and their ability to positively alter gut health and function (Jang et al. 2007; Yan & Kim 2013). A possible alternative for antibiotics as growth promoters are organic acids. Organic acids are weak acids with at least one carboxylic group ($-\text{COOH}$) and a carbon chain having one to seven carbon atoms. According to Canibe et al. (2001) organic acids can positively influence the micro flora in the gastrointestinal tract, thus improving the health of the pigs. Different organisms produce organic acids naturally or they are also produced by the metabolism. Therefore, organic acids are not considered to be harmful in adequate doses (Partanen & Mroz 1999). Previous reports indicate that organic acid supplement in pig diet has beneficial effects in swine performance (Mroz et al. 2006; Wang et al. 2009a) as well as in poultry (Wang et al. 2009b). The positive effects of dietary organic acids supplementation directly on gut health and development, and indirectly on pig health and productivity, may be attributed to various factors, including: (1) antimicrobial activity of non-dissociated organic acids; (2) lowering the pH of digesta particularly in the stomach, aiding protein

digestion; (3) reducing the emptying rate of stomach; (4) stimulating (pancreatic) enzyme production and activity in the small intestine; and (5) providing nutrients to intestinal tissue thereby enhancing mucosal integrity and function (de Lange et al. 2010).

Different combinations of organic acids (Ahmed et al. 2014) and inorganic acids are used increasingly in diets for weaning as well as growing-finishing pigs and sows due to their beneficial and synergistic effects. The combination of organic acid and medium chain fatty acid (MCFA) has been reported to have beneficial effect in the intestinal micro ecology in piglets (Zentek et al. 2013). This characteristic is associated with the much higher antimicrobial strength of the acid undissociated form.

With the development of coating technology, protected acids by encapsulation for targeted delivery to different gut segments have gained considerable attention. Bosi et al. (1999) noted that to control the overgrowth of *Escherichia coli* and other dangerous bacteria in the lower tract of small intestine and hind gut, slowing down the release of the acids added to the diets would be helpful and their findings concluded that supplementation of protected organic acid tended to increase the *Lactobacillus* population in the ileum compared with unprotected organic acids. This indicates that protected organic acids are more effective in the delivery of organic acids to the

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distal ileum, caecum and colon of piglets thereby improving the growth of beneficial micro-organisms and interfering the survival rate of enteric pathogens.

The objective of the present study was to evaluate the impact of novel development of organic acid blends protected by lipid base matrix coating on the growth performance, nutrient digestibility and faecal micro flora in growing pigs.

2. Materials and methods

The experimental protocols describing the management and care of animals were reviewed and approved by the Animal Care and Use Committee of Dankook University.

2.1. Source of organic acid

The matrix coated organic acid used in the current experiment is provided by a commercial company (Morningbio Co., Ltd., Cheonan, Korea). This protected organic acid consists of MCFA and composite organic acids. The active ingredients are 17% fumaric acid, 13% citric acid, 10% malic acid and 1.2% MCFA (capric and caprylic acid) and carrier.

2.2. Experimental animals

A total of 100 crossed [(Landrace × Yorkshire) × Duroc] growing pigs with an initial body weight (BW) of 23.4 ± 1.27 kg were used in a six-week trial. Pigs were randomly allocated into one of four treatments (five replications with five pigs per pen) in a randomly complete block design according to their BW and sex (three barrows and two gilts). Protected organic acids blends were administered by replacing same amount of corn. Dietary treatments were as follows: (1) CON, basal diet; (2) MC1, CON + 0.1% protected organic acid; (3) MC2, CON + 0.2% protected organic acid and (4) MC3, CON + 0.4% protected organic acid. Prior to experimental feeding, pigs were on adaptation diet for seven days. All pigs were allowed *ad libitum* access to feed and water through a self-feeder and nipple drinker throughout the experimental period. The diets used in this experiment were formulated to meet or exceed NRC (2012; Table 1).

2.3. Sampling and measurements

BW was measured at the beginning and at six weeks of the experimental period, and feed consumption was recorded on a pen basis during the experiment to calculate the average daily gain (ADG), average daily feed intake (ADFI) and feed efficiency (G:F). Chromium oxide was added to the diet as an indigestible marker at 0.20% for seven days prior to faecal collection at sixth week for calculation of dry matter (DM), N and energy digestibility. Pooled faecal grab samples were collected at random

Table 1. Composition of basal diets for growing pigs (as-fed basis).

Items	Content
<i>Ingredients (g/kg)</i>	
Corn	553.8
Soybean meal	334.3
Molasses	25.0
Animal fat	53.3
Difluorinated phosphate	19.3
Limestone	7.8
L-lysine HCl	1.7
Trace mineral premix ^a	1.0
Vitamin premix ^b	1.2
Salt	2.0
DL-methionine	0.3
Choline chloride	0.3
<i>Chemical composition</i>	
ME (kcal/kg)	3360
Crude protein (%)	20.00
Lysine (%)	1.30
Calcium (%)	0.90
Phosphorus (%)	0.80
<i>Analysed composition</i>	
GE (kcal/kg)	4112
Crude protein (%)	19.65
Calcium (%)	0.87
Phosphorus (%)	0.76

^aProvided per kg of complete diet: 12.5 mg Mn, 179 mg Zn, 140 mg Cu, 0.5 mg I and 0.4 mg Se.

^bProvided per kg of complete diet: 20,000 IU of vitamin A; 4000 IU of vitamin D3; 80 IU of vitamin E; 16 mg of vitamin K3; 4 mg of thiamine; 20 mg of riboflavin; 6 mg of pyridoxine; 0.08 mg of vitamin B12; 120 mg of niacin; 50 mg of Ca-pantothenate; 2 mg of folic acid and 0.08 mg of biotin.

from two pigs (one gilt and one barrow) in each pen. All feed and faeces samples were stored immediately at -20° C until analysis. Faecal samples were dried at 70° C for 72 h and finely ground to pass through a 1-mm screen. All of the feed and faecal samples were then analysed for DM and N following the procedures outlined by the AOAC (2000). Chromium was analysed using UV absorption spectrophotometry (Shimadzu, UV-1201, Kyoto, Japan) and nitrogen was determined using a Kjeltac 2300 Analyzer (Foss Tecator AB, Hoeganaes, Sweden). The apparent total tract digestibility was calculated according to the method described by Fenton and Fenton (1979) using the following formula: digestibility (%) = $\{1 - [(Nf \times Cd)/(Nd \times Cf)]\} \times 100$, where Nf = nutrient concentration in faeces (% DM), Nd = nutrient concentration in diet (% DM), Cd = chromium concentration in diet (% DM) and Cf = chromium concentration in faeces (% DM).

Gross energy was determined by measuring the heat of combustion in the samples using a Parr 6100 oxygen bomb calorimeter (Parr Instrument Co., Moline, IL, USA).

For *Lactobacillus* and *E. coli* population analysis, fresh faeces taken via massaging the rectum from 2 pigs

Table 2. Effects of protected organic acid supplementation on growth performance in growing pigs.

Items	CON	MC1	MC2	MC3	Standard error	P value	
						Linear	Quadratic
ADG (g)	713 ^b	724 ^{ab}	745 ^a	737 ^{ab}	9	0.04	0.3
ADFI (g)	1586	1590	1584	1588	4.5	0.93	1.0
G/F	0.450 ^b	0.456 ^{ab}	0.471 ^a	0.464 ^{ab}	0.01	0.03	0.4

CON, basal diet; MC1, CON + 0.1% protected organic acid; MC2, CON + 0.2% protected organic acid; MC3, CON + 0.4% protected organic acid.
^{a,b}Means in the same row with different superscripts differ ($p < 0.05$).

per pen at 42 day of the experiment and, respectively, pooled and placed on ice for transportation to the laboratory. The composite faecal sample (1 g) from each pig was diluted with 9 mL of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) and then homogenised. Viable counts of bacteria in the faecal samples were conducted by plating serial 10-fold dilutions (in 1% peptone solution) onto Mac Conkey agar plates (Difco Laboratories, Detroit, MI, USA) and lactobacilli medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany) to isolate the *E. coli* and *Lactobacillus*, respectively. The lactobacilli medium III agar plates were incubated for 48 h at 39°C under anaerobic conditions. The Mac Conkey agar plates were incubated for 24 h at 37°C. The *E. coli* and *Lactobacillus* colonies were counted immediately after removal from the incubator.

2.4. Statistical analyses

All data were subjected to statistical analysis in a randomised complete block design using the GLM procedures (SAS, 1996, SAS Institute Inc., Cary, NC, USA), with the pen serving as the experimental unit. The initial BW was used as a covariate for the ADFI and ADG. Differences between treatments were separated by Duncan's multiple range tests when the treatment effect was observed with the alpha level of 0.05. Before conducting statistical analysis of the microbial counts, the value was transformed logarithmically. Linear and quadratic polynomial contrasts were performed to determine the treatment differences, i.e. effects of inclusion level of 0.1%, 0.2% and 0.4% of protected organic acid blends in the diet.

3. Results and discussion

At the end of six weeks of experimental trial, pigs fed diets containing 0.2% protected organic acid showed greater ADG (linear, $P = 0.04$) and gain/feed (linear, $P = 0.03$) than pigs fed the control and other treatment diet (Table 2). The dietary supplementation with protected organic acid did not affect ($P > 0.05$) nutrient digestibility (Table 3).

The present finding shows that when pigs are supplemented with feed containing 0.2% protected organic acid blends, their a ADG and feed efficiency improved compared with the pigs fed control diet. Consistent to our findings some researchers have shown positive effects with single or blends of dietary acidifiers. For instance, supplementation of single acidifier such as formic or sorbic improved growth rate and feed efficiency (Overland et al. 2008). Likewise, Li et al. (2008) reported that blends of organic acids such as botanic, fumaric and benzoic acid at the rate of 5 g/kg in the diet of weanling piglet numerically improved growth performance. In contrast other reports indicate nothing or negative responses with single acidifiers such as fumaric, citric or formic acid (Radecki et al. 1988; Manzanilla et al. 2004) or blend of acidifiers such as formic acid, lactic acid and volatile fatty acids (Lee et al. 2007). The inconsistent results and highly variable responses may be due to several factors such as stage of growth, diet complexity, type of acid, inclusion level of acid and health status of pig. The enhancement in the performance of pigs in the current study could be due to increase in beneficial bacteria and reduction of pathogenic bacteria in gastrointestinal tract. Thus, improvement in growth performances with the addition of organic acids in the diet is due to the antimicrobial effect which is also

Table 3. Effects of protected organic acids supplementation on nutrient digestibility in growing pigs.

Items (%)	CON	MC1	MC2	MC3	Standard error	P value	
						Linear	Quadratic
Dry matter	73.93	73.83	74.6	73.55	0.3	0.27	0.11
Nitrogen	72.06	71.52	72.03	71.24	0.49	0.16	0.80
Energy	71.32	71.31	71.83	71.27	0.33	0.56	0.38

CON, basal diet; MC1, CON + 0.1% protected organic acid; MC2, CON + 0.2% protected organic acid; MC3, CON + 0.4% protected organic acid.

Table 4. Effects of protected organic acid supplementation on faecal micro flora in growing pigs.

Items (log ₁₀ cfu/g)	CON	MC1	MC2	MC3	Standard error	P value	
						Linear	Quadratic
<i>Lactobacillus</i>	7.26 ^b	7.31 ^b	7.47 ^a	7.38 ^{ab}	0.04	0.01	0.2
<i>E. coli</i>	6.27	6.19	6.05	6.02	0.10	0.20	0.9

CON, basal diet; MC1, CON + 0.1% protected organic acid; MC2, CON + 0.2% protected organic acid; MC3, CON + 0.4% protected organic acid.
^{a,b}Means in the same row with different superscripts differ ($p < 0.05$).

demonstrated by Kirchgessner et al. (1992). The improvement in growth performance with efficient feed conversion rate has its significance in healthy pig production which would eventually contribute to meet the increasing demand of meat worldwide.

In the present study DM, nitrogen and energy digestibility were not affected with organic acid supplements which are consistent with the findings of Kil et al. (2006) and Radecki et al. (1988) who indicated that DM and CP were not improved by organic acid such as formic, fumaric or lactic acid. In contrast, other researcher observed favourable effect on nutrient digestibility with organic acid supplementation such as 2% benzoic acid in the diet of lactating sows (Kluge et al. 2010). The variation in results could be due to age of animal, composition of diet and amount of organic acid supplemented (Ravindran & Kornegay 1993). The response to organic acid supplementation is more pronounced in young pigs around weaning because of immature digestive system. The dosage level of organic acids in the present experiment might be too low to affect nutrient digestibility. However, no adverse effect on digestibility was observed with the inclusion of organic acids in the diet.

An increased ($P < 0.05$) in faecal *Lactobacillus* concentration as well as linear effect (linear, $P = 0.01$) was observed with 0.2% protected organic acid diets (Table 4). The *E. coli* concentration was not affected ($P > 0.05$) by dietary treatments.

The present study shows the linear increase in *Lactobacillus* population in the faeces obtained from pigs fed diet supplemented with protected organic acid compared with control. However, *E. coli* population was not affected significantly with organic acid supplementation though the population was numerically lower than the control. In line with our finding, Li et al. (2008) and Ahmed et al. (2014) also reported that blends of organic acid supplementation led to reduced *E. coli* and increased *Lactobacilli* concentration in weaned piglets. The increase in *Lactobacillus* population benefits intestinal function by reducing the survival rate of enteric pathogens due to the low pH resulting from the acid produced by the fermentation of *Lactobacillus*. As the microbial pathogenic population gets reduced, the metabolic need of the microbes is reduced and the availability of dietary energy and nutrients

to the host animal is increased, leading to enhanced growth rate and enhanced feed efficiency.

4. Conclusion

In conclusion, our results suggest that 0.2% protected organic acid supplementation enhanced growth performance and improved gut microbial population without any adverse effect in nutrient digestibility in growing pigs. Hence, organic acids blends can be considered as potential alternative to antibiotics in order to improve performance in growing pigs.

Disclosure statement

No potential conflict of interest was reported by the authors.

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